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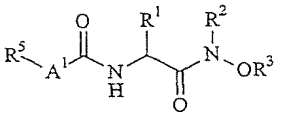
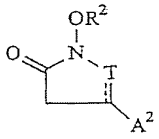
(54) Title: HIF HYDROXYLASE INHIBITORS



(A)



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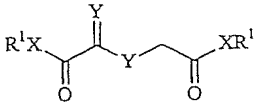
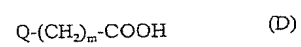
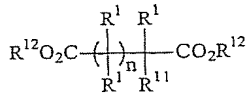


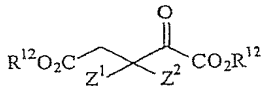
(C)



(D)



(E)



(F)

(57) Abstract: The invention provides a compound of one of the formulae (A), (B), (C), (D), (E), (F) as herein defined, or a salt thereof, for use in the treatment of a condition associated with increased or decreased HIF levels or activity, or a condition in which an increase or decrease in HIF levels or activity may be beneficial.

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HIF HYDROXYLASE INHIBITORS

Field of Invention

The present invention relates to compounds which modulate 2OG (2-oxoglutarate) dependent oxygenases, in particular prolyl hydroxylases. These may be useful as modulators of HIF (hypoxia inducible factor) alpha (HIF-α) prolyl hydroxylase.

Background of Invention

The transcription factor HIF (hypoxia inducible factor) system is a key regulator of responses to hypoxia, occupying a central position in oxygen homeostasis in a wide range of organisms. A large number of transcriptional targets have been identified, with critical roles in angiogenesis, erythropoiesis, energy metabolism, inflammation, vasomotor function, and apoptotic/proliferative responses. The system is essential for normal development, and plays a key role in pathophysiological responses to ischaemia/hypoxia. HIF is also important in cancer, in which it is commonly upregulated, and has major effects on tumour growth and angiogenesis. The HIF DNA binding complex consists of a heterodimer of α and β subunits. Regulation by oxygen occurs through hydroxylation of the α-subunits, which are rapidly destroyed by the proteasome in oxygenated cells. This involves binding of HIF-α-subunits by the von Hippel-Lindau tumour suppressor protein (pVHL), with pVHL acting as the, or part of the, recognition component for a ubiquitin ligase that promotes ubiquitin dependent proteolysis through interaction with a specific sequence or sequences in HIF-α-subunits. In hypoxia, this process is suppressed, so stabilizing HIF-α and permitting transcriptional activation via the HIF α, β.

Disclosure of the Invention

In our British Application No. 0118952.1 we disclose a polypeptide comprising:

(a) the amino acid sequence of SEQ ID NO: 2, 4 or 6 having HIF hydroxylase activity;

(b) a variant thereof having at least 60% identity to the amino acid

sequence of SEQ ID NO: 2, 4 or 6 and having hydroxylase activity; or

(c) a fragment of either thereof having HIF-α hydroxylase activity.

Preferably, the polypeptides have prolyl hydroxylase activity and require Fe(II) for activity.

They are related by sequence to non-haem oxygenases for which crystal structures are known, e.g. proline-3-hydroxylase (Clifton et al, Eur. J. Biochem, 2001, 268, 6625-6636).

It also discloses polynucleotides which encode the polypeptides as well as expression vectors comprising the polynucleotide and antibodies capable of specifically binding the polypeptide. We also disclose assays for identifying modulators of the activity of the HIF hydroxylase as well as the use of modulators such as inhibitors of the activity of the peptides in the treatment of a condition or disease associated with altered HIF levels with respect to healthy (or normal) levels, and the treatment of conditions where an alteration in the HIF levels or activity would be beneficial.

Inhibitors of the 2-OG dependent enzyme collagen prolyl-4-hydroxylase (CPH) are well known in the art and have been previously proposed for use in the treatment of lung fibrosis, skin fibrosis (scleroderma), atherosclerosis and other conditions associated with collagen biosynthesis. Inhibitors of para-hydroxyphenylpyruvate oxygenase (a non-haem oxygenase employing ferrous iron as a co-factor) such as triketones are used as herbicides (Lee D. et al (1998) Pestic. Sci. 54(4) 377-384). We have disclosed that certain of these CPH inhibitors (and other components) also inhibit the biological (i.e. HPH) activity of an PHD polypeptide. A CPH inhibitor or modified CPH inhibitor which inhibits the biological activity of an PHD polypeptide may be used in the treatment of a condition associated with reduced or suboptimal HIF levels or activity, or a condition in which an increase in HIF levels or activity may be beneficial, for example ischaemia, wound healing, auto-, allo-, and xeno- transplantation, systemic high blood pressure, cancer, inflammatory disorders, and metabolic disorders.

Various methods and uses of modulators which inhibit, potentiate, increase or stimulate hydroxylation of HIF-α by an PHD polypeptide are disclosed. The purpose

of disruption, interference with or modulation of the hydroxylation of HIF-1α by a PHD polypeptide may be to modulate cellular functions such as angiogenesis, erythropoiesis, energy metabolism, inflammation, matrix metabolism, vasomotor function, and apoptotic/proliferative responses and pathophysiological responses to ischaemia/hypoxia, all of which are mediated by HIFα as discussed above.

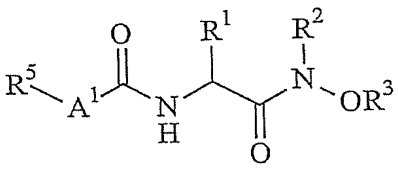
Compounds which modulate 2OG oxygenases, in particular CPH may be useful as modulators of HIF prolyl hydroxylase, or may be used as 'lead' compounds which may be modified and/or optimised to develop modulators of HIF prolyl hydroxylase, in particular selective modulators are described.

Some of these compounds generally possess the formula:

$$R^1A^*B^*C^*D^*(R^2)_y \qquad (A)$$

where the group R^1 is capable of forming an electrostatic interaction with the sidechain of the arginine residue which, together with other residues, binds the 5-carboxylate of 2-oxoglutarate during catalysis, A*B is a chain of two atoms which are, independently, carbon, oxygen, nitrogen or sulphur, which chain can be functionalised, y is 0 or 1 and C*D is a chain of two atoms which are, independently, carbon, oxygen, nitrogen, or sulphur, which chain can be functionalised, A, B, C and D being linked to one another by a single and/or double and/or triple bond such that when y is 0 or 1at least one of the atoms of which is capable of chelating with a metal group and when y is 1 said chain is attached to R^2 which is capable of chelating with a metal group. Generally at least one of A, B, C and D is not carbon. Typical chains include C-N-C-C and C-O-C-C and C-C-C=O. The chain atoms can form part of a ring.

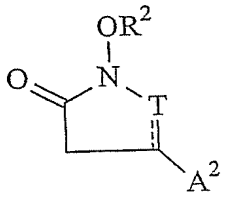
New classes of modulators of HIF prolyl hydroxylase have been found, according to the present invention. These possess the following formulae (A) to (F)



(A)

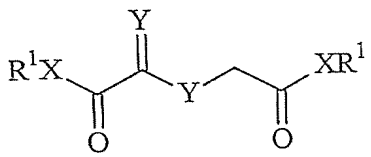
where each of R^1 and R^5 is independently H, OH, SH, a branched or straight C_1 to C_6

alkyl chain optionally containing 1 or more eg. 2 N, S, O or P chain atoms, especially methyl, which can be functionalised, any amino acid side chain, such as alanine, phenylalanine, valine and glutamic acid, a 4 to 7 membered heterocyclic ring optionally containing 1 or 2 N, S, O or P atoms or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms which may be fused to another ring or a said alkyl chain substituted by a said aromatic ring, such as aryloxy alkyl, A^1 is CH_2 or O, and each of R^2 and R^3 is independently be H, OH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more eg. 2 N, S, O or P chain atoms which can be functionalised, optionally with 1, 2, 3, 4 or 5 halo substitutions, a 4 to 7 membered heterocyclic ring optionally containing 1 or 2 N, S, O or P atoms, or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms which may be fused to another ring or a said alkyl chain substituted by a said aromatic ring,



(B)

wherein R^2 is as defined above, - - - is a single bond and T is CH_2 or C=O, or - - - is a double bond and T is CH; A^2 is H or $-XCO_2R^4$; X is a single bond or a branched or straight C_1 to C_6 alkyl chain, optionally containing 1 or more eg. 2 N, S, O or P chain atoms and optionally substituted by eg. halo, OH, NHR^2 or $NHCOR^4$ where R^2 and R^4 are as defined above and R^4 represents H, a branched or straight chain C_1 to C_6 alkyl group optionally containing 1 or more eg. 2 N, S, O or P chain atoms, a 4 to 7 membered heterocyclic ring, optionally containing 1 or 2 N, S, O or P atoms, or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms, which may be fused to another ring, or a salt thereof,

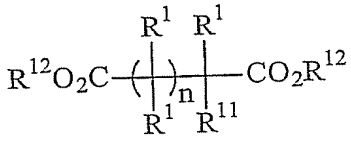


(C)

where each X which may be the same or different is NH, NR" , where R" is OH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more eg. 2 N, S, O or P chain atoms which can be functionalised, or O i.e. XR^1 is typically OH or O-alkyl having a branched or straight C_1 to C_6 alkyl chain, especially MeO, each Y, which may be the same or different, is O or S and each R^1, which may be the same or different, is as defined above,

$$Q\text{-}(CH_2)_m\text{-}COOH \qquad (D)$$

where m is 0 or 1, Q represents $(R^1R^6)_xZ$ where x is 0, 1 or 2, R^1 is as defined above and R^6 is as defined for R^1, and Z is $P{=}O(OH)_2$, $B(OH)_2$ or SO_3H, or a salt thereof, typically a sodium salt, or

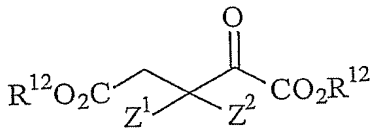


(E)

where each R^1, which are the same or different, is as defined above; R^{11} represents OH or R^{10} NH where R^{10} is HO, R^1CO or $HOOC(X)_x$ wherein R^1 is as defined above, x is 0 or 1 and X is R^1R^1C wherein each R^1, which are the same or different, is as defined above; or R^{10} is an amino acid residue H_2N (R^1R^1C) CO - wherein each R^1, which are the same or different, is as defined above; n is 1 or 2 and R^{12} is H or straight or branched C_1 to C_6 alkyl; or a salt thereof. Typically X is CH_2 or CHOH.

Another aspect of the invention concerns analogues of 2-oxoglutarate that act as improved (relative to 2-oxoglutarate) co-substrates for the HIF hydroxylases. Such a compound is 3-fluoro 2-oxoglutarate. Assays in which this compound replaces 2-oxoglutarate demonstrate a higher level of HIF hydroxylation than observed when using 2-oxoglutarate under analogous conditions.

These analogues possess the formula:



(F)

wherein each of Z^1 and Z^2 is independently hydrogen, SH or an electron withdrawing group such as halogen, preferably fluorine, or alkoxy such as methoxy, and R^{12} is as defined above, or a salt thereof. Preferably one of Z^1 and Z^2 is hydrogen and the other is fluorine (3-F-2-OG).

Accordingly the present invention provides a compound of formula (A) to (F) for use in the treatment of a condition associated with increased or decreased HIF levels or activity, or a condition in which an increase or decrease in HIF levels or activity may be beneficial, as well as the use of a compound of formula (A) to (F) in the manufacture of a medicament for the treatment of such a condition.

The said alkyl groups and chains are typically functionalised by alcohol, fluorine, thiol, a carboxylic acid, phosphonic or phosphinic acid, sulphonic acid or other chelating group, in the case of the chains typically via an alkyl group.

In the formulae described herein, a branched or straight C_1 to C_6 alkyl chain may be a methyl, ethyl, propyl, butyl, iso-butyl, *tert*-butyl, pentyl, neopentyl, *tert*-pentyl or a primary, secondary or tertiary hexyl group. Hetero atoms such as O, S, N and P may replace one or more of the carbon atoms. Preferably the alkyl groups are methyl, the preferred heterocyclic rings are pyrolidine and tetrahydropyran and the preferred aromatic rings are benzene, naphthalene and pyridine.

Typically, each of R^1 and R^5 is independently H, OH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more N, S, O or P chain atoms, which can be functionalised, any amino acid side chain, a 4 to 7 membered heterocyclic ring optionally containing 1 or 2 N, S, O or P atoms or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms which may be fused to another ring or a said alkyl chain substituted by a said aromatic ring.

Typically, A^1 is CH_2.

Typically, A^2 is $-XCO_2R^4$.

Typically, R^{11} represents R^{10} NH where R^{10} is R^1CO or $HOOC(X)_x$ wherein

R^1 is as defined above, x is 0 or 1 and X is R^1R^1C wherein each R^1, which are the same or different, is as defined above; or R^{10} is an amino acid residue H_2N (R^1R^1C) CO- wherein each R^1, which are the same or different, is as defined above.

Typically, each of Z^1 and Z^2 is independently hydrogen or an electron withdrawing group.

Typically, in the compounds of formula (F), R^{12} is H. Alternatively, R^{12} may be straight or branched C_1 to C_6 alkyl.

The compounds of formula (A) are hydroxamates. Preferred compounds include those where R^5 is aryloxyalkyl, especially oxyloxymethyl such as phenyloxymethyl or phenylalkyloxymethyl, especially benzyloxymethyl or substituted benzyloxymethyl such as p-hydroxy benzyloxymethyl and/or where R^2 and/or R^3 is $HOCH_2$.

Typical compounds include N-phenoxy-acetyl-(L)-alanine-hydroxamide (Is41) and the corresponding (D) isomer (Is43) as well as the corresponding tyrosine derivatives (Is44 and 45) and L- and D-phenylglycine derivatives (Is46 and 47), along with benzo hydroxamic acid and N-phenoxyacetyl-D-phenylalanine hydroxamic acid (Is42).

These compounds can generally be prepared following the method of Walter et al., Tetrahedron 1997, 53, 7275-7290 and Biorg. Chem 1999, 27, 35-40.

The compounds of formula (B) are cyclic hydroxamates. Preferred compounds are those where X is a single bond or methyl and/or R^2 is H or phenylalkyl, especially benzyl and/or R^4 is H or methyl. Typical compounds include (1-hydroxy-2, 5-dioxo-pyrrolidin-3-yl) acetic acid (Is52), (1-hydroxy-2, 5-dioxo-pyrrolidin-3-yl) carboxylic acid (ANU 2) and its N-benzoyloxy derivative (ANU 1) along with (1-benzyloxy-2, 5-dioxo-pyrrolidin-3-yl) acetic acid (Is50) and the corresponding methyl ester (Is64), and N-hydroxy succinimide (C1). Note that Is52 (R^2=H, T=C=O, X=CH_2 R^4=H) is highly active reflecting its structural analogy with 2-oxoglutarate. These compounds can be prepared using the general procedure of Schlicht et al. (US 4446038).

The compounds of formula (C) are analogues of 2-oxoglutarate or oxalyl derivatives of hydroxyacetate and mercapto acetic acid. Preferred compounds

include those where X is O and/or R^1 is H or methyl. Typical compounds include dimethyl oxalylglycolate (Is10) as well as its free acid (Is14) and dimethyl oxalylthioglycolate (Is11). These compounds can be prepared following Franklin et al., J. Med. Chem 1992, 35, 2652-2658 or Kwon et al., J. Am. Chem. Soc. 1989, 111, 1854-1860.

The compounds of formula (D) are carboxylic acids which possess a phosphonic, sulphonic or boronic acid group as well as salts of these. Typically R' and R^6 are hydrogen. Preferred compounds include the phosphoric acids where x is 0, 1 or 2 (C3, 4 and 5, respectively) as well as disodium 3-sulpho-propionate (Is63) and its free acid, and 3-borono-propionic acid (Is62).

The compounds of formula (E) are N-acylated amino acids or polycarboxylic acids. Typical compounds are those where R^1 is H, and/or R^{12} is H or ethyl. When R^{11} represents $R^{10}NH$ the compounds are typically dipeptides such that R^{10} is an acyl group of a natural amino acid such as glycine. Typical preferred such compounds include Asp-Gly (C18), cyclo (Asp-Gly) (C19), beta-Asp-Gly (C20), Glu-Gly (C21) and Z-Glu-Gly (C22). Other typical compounds include those where R^{10} is acetyl or benzoyl such as the N-acetylated derivatives of L-aspartic acid (C6) and of L-glutamic acid (C7) i.e. R^{10} is acetyl and N-benzoylated derivatives of glutamic acid (C15 and Is90) i.e. R^{10} is benzoyl. Other typical compounds include those where R^{11} is -NHOH such as diethyl 2-(hydroxylamino)-glutarate (Is51 being the racemic form of this compound) and those where R^{11} is OH such as 2-hydroxyglutaric acid (Is57). When R^{11} is HOOC(X)x, X is especially CH^2 or CHOH. The compounds are typically citric acid (C12), tricarballylic acid (C13) and succinic acid as well as the tri-methyl ester of ethane tricarboxylic acid (Is72).

The compounds of formula (F) are analogues of 2-oxoglutarate. Preferred compounds include 3-fluoro-2-oxoglutarate compounds (i.e. Z^1 is H and Z^2 is F) such as 3-fluoro-2-oxoglutaric acid (Is18) and the corresponding dimethyl ester (Is19).

The compounds which are acids can be present in the form of salts, such as sodium salts.

For therapeutic treatment, the compound may be used in combination with any other active substance, e.g. for anti-tumour therapy another anti-tumour

compound or therapy, such as radiotherapy or chemotherapy.

Generally, the modulator is provided in an isolated and/or purified form, i.e. substantially pure. This may include being in a composition where it represents at least about 90% active ingredient, more preferably at least about 95%, more preferably at least about 98%. Any such composition may, however, include inert carrier materials or other pharmaceutically and physiologically acceptable excipients, such as those required for correct delivery, release and/or stabilisation of the active agent. As noted below, a composition according to the present invention may include in addition to a modulator compound as disclosed, one or more other molecules of therapeutic use, such as an anti-tumour agent.

In general they take the form of compositions wherein the compound is in a mixture with a pharmaceutically acceptable carrier or diluent. The carrier may be liquid, e.g. saline, ethanol, glycerol and mixtures thereof, or solid, e.g. in the form of a tablet, or in a semi-solid form such as a gel formulated as a depot formulation or in a transdermally administerable vehicle, such as a transdermal patch. The modulator compound or composition comprising it may be formulated as the coating of a coated stent.

The invention further provides a method of treatment which includes administering to a patient compound as defined above. Exemplary purposes of such treatment are discussed elsewhere herein.

The therapeutic/prophylactic purpose of such a method or use may be the modulation of the level of HIFα in a cell by modulation, e.g. disruption or interference, of the hydroxylation of HIFα, which may occur for example at proline 402, 564 or other proline residue. Hydroxylation of HIFα promotes pVHL binding which leads to ubiquitin dependent proteolysis of HIFα as described above.

The therapeutic/prophylactic purpose may be related to the treatment of a condition associated with reduced or suboptimal or increased HIF levels or activity, or conditions where an alteration in HIF levels or activity may be beneficial such as:
(i) ischaemic conditions, for example organ ischaemia, including coronary, cerebrovascular and peripheral vascular insufficiency. The therapy may be applied in two ways; following declared tissue damage, e.g. myocardial infarction (in order to

limit tissue damage), or prophylactically to prevent or ameliorate ischaemia, e.g. promotion of coronary collaterals in the treatment of angina.

(ii) wound healing and organ regeneration.

(iii) auto-, allo-, and xeno- transplantation.

(iv) systemic blood pressure.

(v) cancer; HIFα is commonly up-regulated in tumour cells and has major effects on tumour growth and angiogenesis.

(vi) inflammatory disorders.

(vii) pulmonary arterial blood pressure, neurodegenerative disease.

(viii) metabolic disorders, e.g. diabetes.

Modulating HIF prolyl hydroxylase activity in a person, an organ, or a group of cells may be exploited in different ways to obtain a therapeutic benefit:

(a) Non cell autonomous: The HIF system is used by cells to influence the production of substances which signal to other cells. These signals may then have effects at (i) a distant site (for example erythropoietin acts on the bone marrow) or (ii) locally (angiogenic growth factors increase the local formation of blood vessels). Manipulating non cell autonomous behaviour via altering hydroxylase activity is therefore useful in the treatment of anaemia, and local ischaemia, for example in the eye, brain, heart and limbs. Many other signals that are involved in aspects of physiological homeostasis may be, or are known to be, adjusted by HIF activation. Consequently altering HIF prolyl hydroxylase activity may be used to potentiate or initiate a helpful response for a therapeutic benefit, or to prevent or ameliorate a harmful response. For example, this approach can be used to alter appetite, or blood pressure in the systemic or pulmonary beds.

(b) Cell autonomous: the HIF system is also used by cells to regulate cellular metabolism, and decisions concerning differentiation, proliferation and apoptosis. Therefore manipulating the HIF system can be used to alter the viability and behaviour of cells. An increase in cell viability can be achieved by increasing HIF activation, for example in an ischaemic tissue. This approach can also be used in improving pancreatic beta cell viability as a way of ameliorating diabetes, or of improving the viability or function of a group or groups of neurons in Parkinson's

disease, motorneurone disease or forms of dementia. In a different approach, the HIF signal can be manipulated to prevent a group of cells proliferating, or to promote its death or differentiation. For example transient activation of the HIF system in a malignant tumour can be used to provoke death of a substantial number of tumour cells.

Pharmaceutical Compositions

In various further aspects, the present invention thus provides a pharmaceutical composition, medicament, drug or other composition for such a purpose, the composition comprising one or more compounds of formulae (A) to (F), or derivatives thereof, the use of such an composition in a method of medical treatment, a method comprising administration of such a composition to a patient, e.g. for treatment (which may include preventative treatment) of a medical condition as described above, use of such an agent compound or substance in the manufacture of a composition, medicament or drug for administration for any such purpose, e.g. for treatment of a condition as described herein, and a method of making a pharmaceutical composition comprising admixing such an agent, compound or substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

The agent may be used as sole active agent or in combination with one another or with any other active substance, e.g. for anti-tumour therapy another anti-tumour compound or therapy, such as radiotherapy or chemotherapy.

Whatever the agent used in a method of medical treatment of the present invention, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

An agent or composition may be administered alone or in combination with

other treatments, either simultaneously or sequentially dependent upon the condition to be treated, e.g. as described above.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous. The compositions will typically be sterile.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Liposomes, particularly cationic liposomes, may be used in carrier formulations. Examples of techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

The substance or composition may be administered in a localised manner to a particular site or may be delivered in a manner in which it targets particular cells or tissues, for example using intra-arterial stent based delivery.

Targeting therapies may be used to deliver the active substance more specifically to certain types of cell, by the use of targeting systems such as antibody

or cell specific ligands. Targeting may be desirable for a variety of reasons, for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

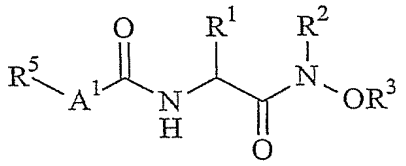
The following Examples further illustrate the present invention.

Example 1

In vitro screening of potential inhibitors of HIF modification was performed using a capture assay. A Gal/HIF-1α/VP16 fusion protein expressing HIF-1α residues 549-582 was prepared by IVTT (see British Application No. 0118952.1) and used as a substrate in the assay. The unlabelled substrate was immunopurified on beads, washed, and aliquots incubated in the presence of RCC4 cell extract, with 100μM $FeCl_2$ and 2mM of the potential inhibitor. The inhibitors were either dissolved in DMSO or Tris as indicated. Controls, where no inhibitor but the equivalent amount of DMSO or Tris was added, were also performed. After washing, the beads were assayed for their ability to interact with 35-S labelled pVHL IVTT. Hydroxylation of the fusion protein by HIF hydroxylase present in the cell extract leads to the ability to capture the labelled pVHL and the amount of labelled protein bound to the fusion protein can then be measured to determine relative HIF hydroxylase activity. Figures 1 to 5 show HIF hydroxylase activity in the presence of a particular inhibitor relative to that seen in the absence of the inhibitor (the DMSO/Tris control).

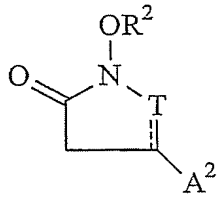
CLAIMS

1. A compound of one of the formulae



(A)

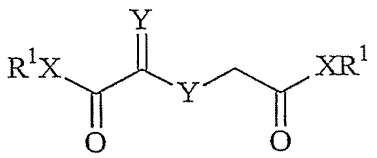
where each of R^1 and R^5 is independently H, OH, SH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more N, S, O or P chain atoms, which can be functionalised, any amino acid side chain, a 4 to 7 membered heterocyclic ring optionally containing 1 or 2 N, S, O or P atoms or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms which may be fused to another ring or a said alkyl chain substituted by a said aromatic ring, A^1 is CH_2 or O, and each of R^2 and R^3 is independently H, OH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more N, S, O or P chain atoms which can be functionalised, optionally with 1, 2, 3, 4 or 5 halo substitutions, a 4 to 7 membered heterocyclic ring optionally containing 1 or 2 N, S, O or P atoms, or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N, O or S atoms which may be fused to another ring or a said alkyl chain substituted by a said aromatic ring,



(B)

wherein R^2 is as defined above, - - - is a single bond and T is CH_2 or C=O, or - - - is a double bond and T is CH; A^2 is H or $-XCO_2R^4$; X is a single bond or a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more N, S, O or P chain atoms, and optionally substituted by halo, OH, OR^4, NRR^2 or $NHCOR^4$ where R^2, R^3 and R^4 are as defined above and R^4 represents H, a branched or straight chain C_1 to C_6 alkyl group, a 4 to 7 membered heterocyclic ring, optionally containing 1 or 2 N, S, O or P atoms, or a 5 or 6 membered aromatic ring, optionally containing 1 or 2 N,

O or S atoms, which may be fused to another ring,

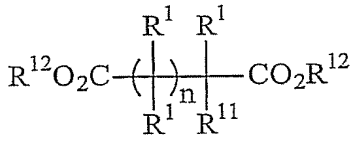


(C)

where each X which may be the same or different is NH, NR" , where R" is OH, a branched or straight C_1 to C_6 alkyl chain optionally containing 1 or more eg. 2 N, S, O or P chain atoms which can be functionalised, or O, each Y, which may be the same or different, is O or S and each R^1, which may be the same or different, is as defined above,

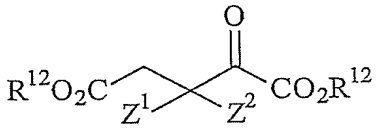
$$Q\text{-}(CH_2)_m\text{-}COOH \qquad (D)$$

where m is 0 or 1, Q represents $(CR^1R^6)_xZ$ where x is 0, 1 or 2, R^1 is as defined above and R^6 is as defined for R^1 and Z is $P{=}O(OH)_2$, $B(OH)_2$ or SO_3H, or a salt thereof, or



(E)

where each R^1, which are the same or different, is as defined above; R^{11} represents OH or R^{10} NH where R^{10} is HO, R^1CO or $HOOC(X)_x$ wherein R^1 is as defined above, x is 0 or 1 and X is R^1R^1C wherein each R^1, which are the same or different, is as defined above; or R^{10} is an amino acid residue H_2N (R^1R^1C) CO- wherein each R^1, which are the same or different, is as defined above; n is 1 or 2 and R^{12} is H or straight or branched C_1 to C_6 alkyl; or a salt thereof,



(F)

wherein each of Z^1 and Z^2 is independently hydrogen, SH or an electron withdrawing group, and R^{12} is as defined above, or a salt thereof for use in the treatment of a

condition associated with increased or decreased HIF levels or activity, or a condition in which an increase or decrease in HIF levels or activity may be beneficial.

2. A compound according to claim 1 which is of formula (A) where R^1 is aryloxymethyl, R^2 is hydrogen and R^3 is hydrogen.

3. A compound according to claim 2 wherein R^1 is phenyloxymethyl, benzyloxymethyl or p-hydroxybenzylmethyl.

4. A compound according to claim 1 of formula (B) where X is a single bond or methyl, R^2 is H or phenyl alkyl and R^4 is H or methyl.

5. A compound according to claim 1 of formula (C) wherein X is O and R^1 is H or methyl.

6. A compound according to claim 1 of formula (D) wherein x is 1, and R^1 and R^6 are both hydrogen.

7. A compound according to claim 1 of formula (E) wherein R^1 is H and R^{12} is H or ethyl.

8. A compound according to claim 7 wherein R^{11} represents R^{10} NH and R^{10} is an acyl group of a natural amino acid, acetyl or benzoyl.

9. A compound according to claim 7 wherein R^{11} is HOOC(X)x.

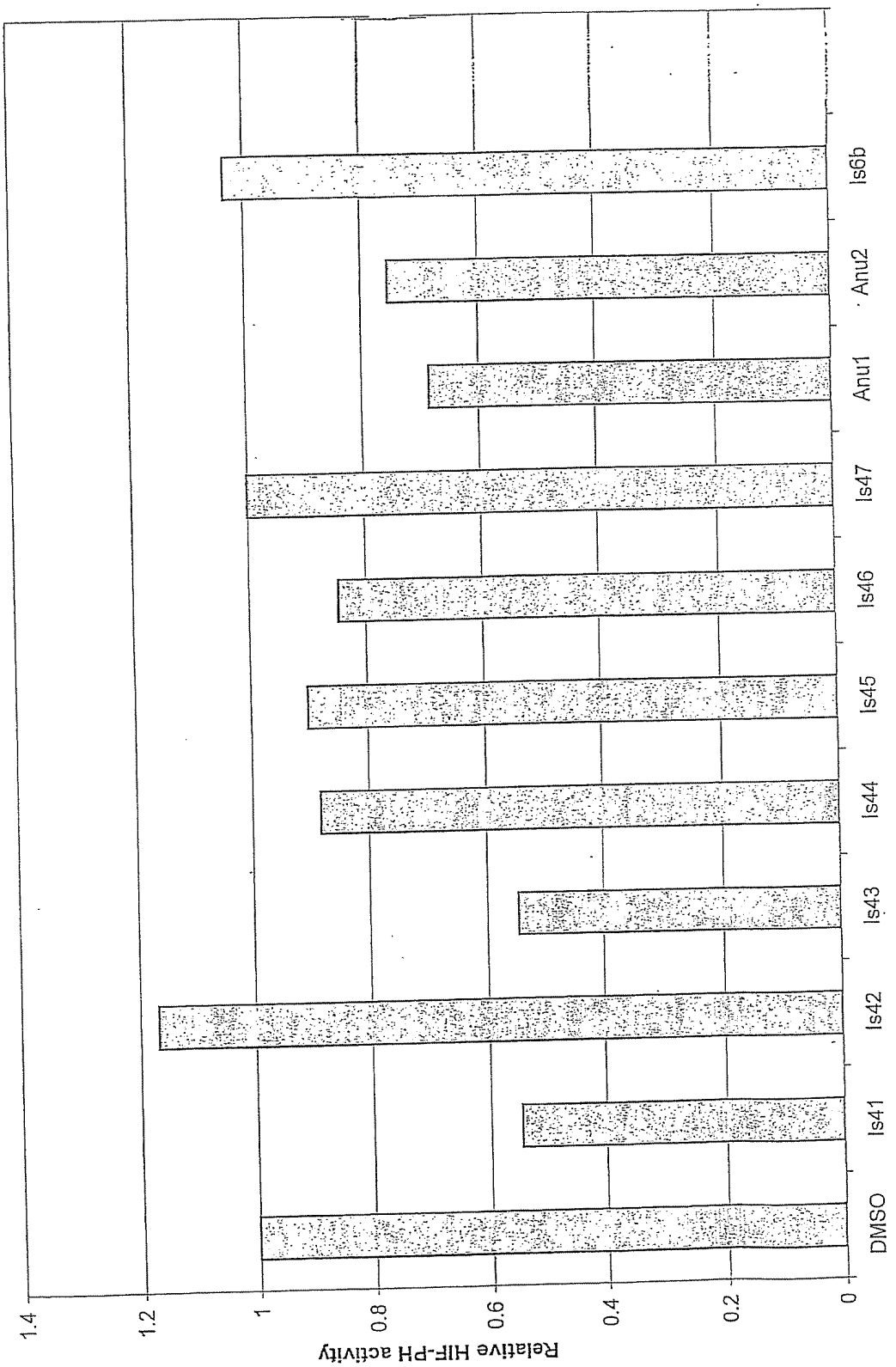
10. Use of a compound as defined in claim 1 in the manufacture of a medicament for the treatment of a condition associated with increased or decreased HIF levels or activity, or a condition in which an increase or decrease in HIF levels or activity may be beneficial.

11. Use according to claim 10 wherein the compound is as defined in any one of claims 2 to 9.

12. A pharmaceutical composition which comprises a compound as defined in claim 1 together with a pharmaceutically acceptable excipient vehicle or carrier.

13. A composition according to claim 12 wherein the compound is as defined in any one of claims 2 to 9.

Figure 1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0.4
0.6
0.8
1
1.2
1.4
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Anu1
Anu2
Is6b

Figure 2

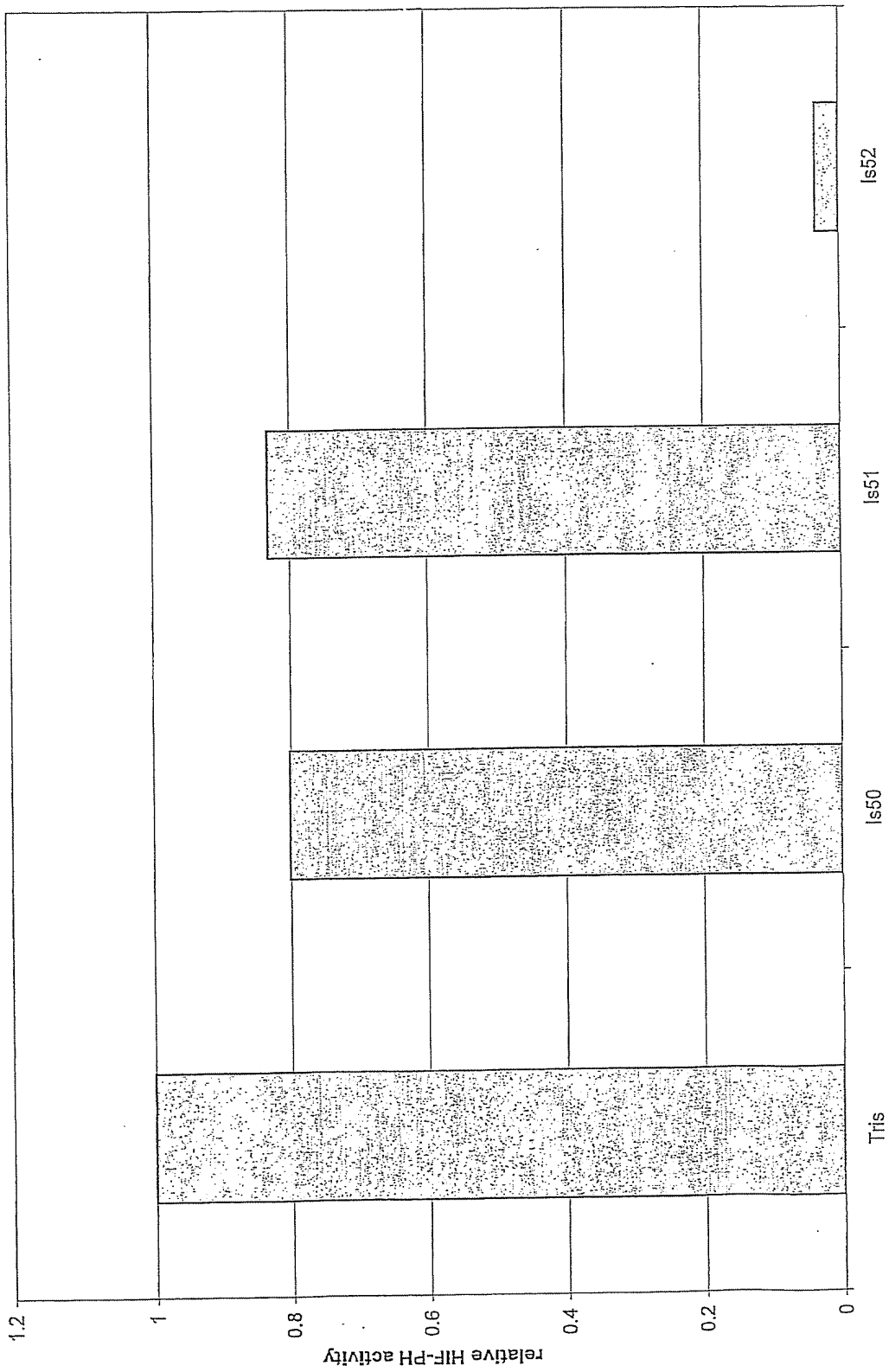
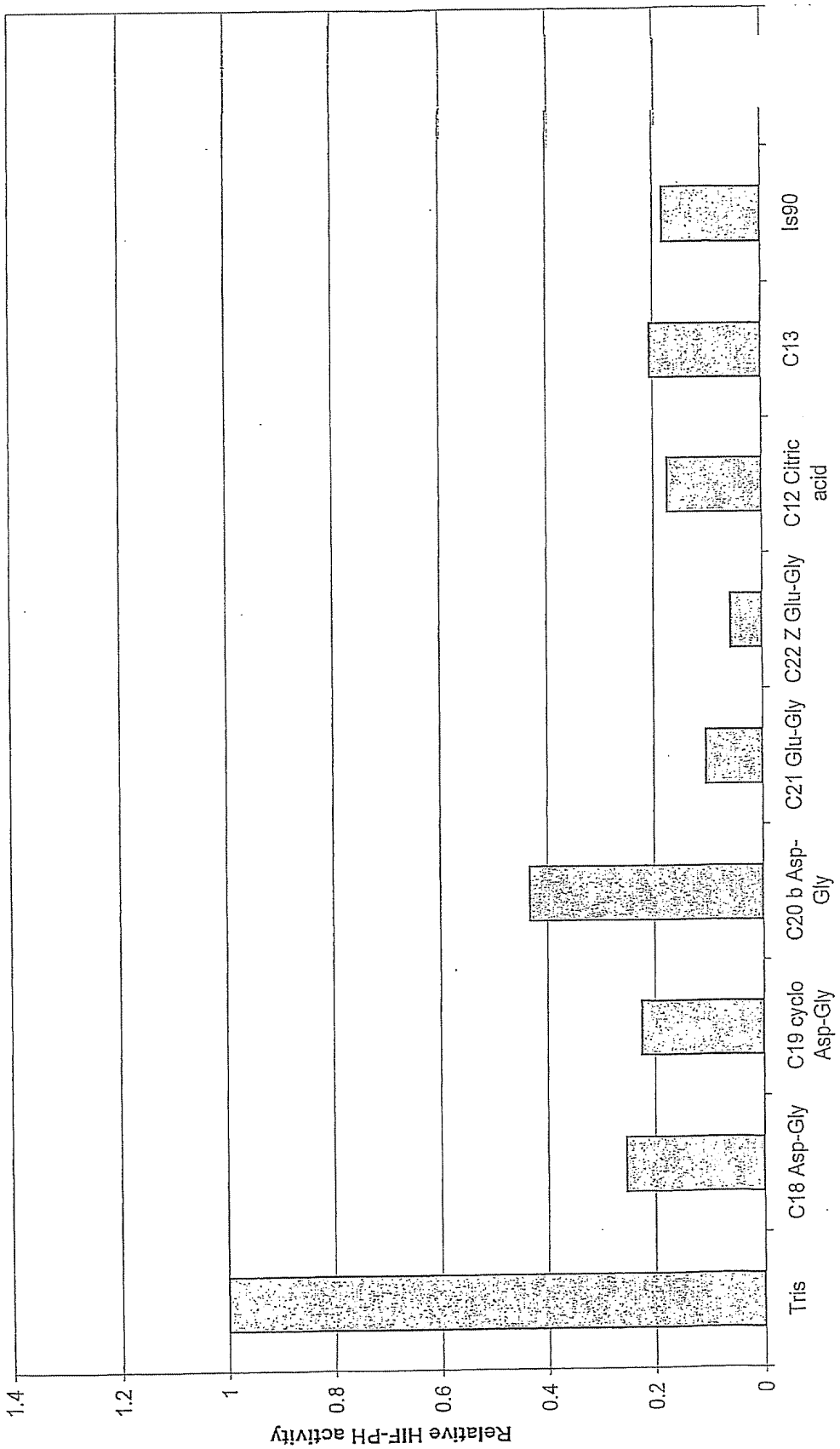


Figure 3


Relative HIF-PH activity
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1.2
1
0.8
0.6
0.4
0.2
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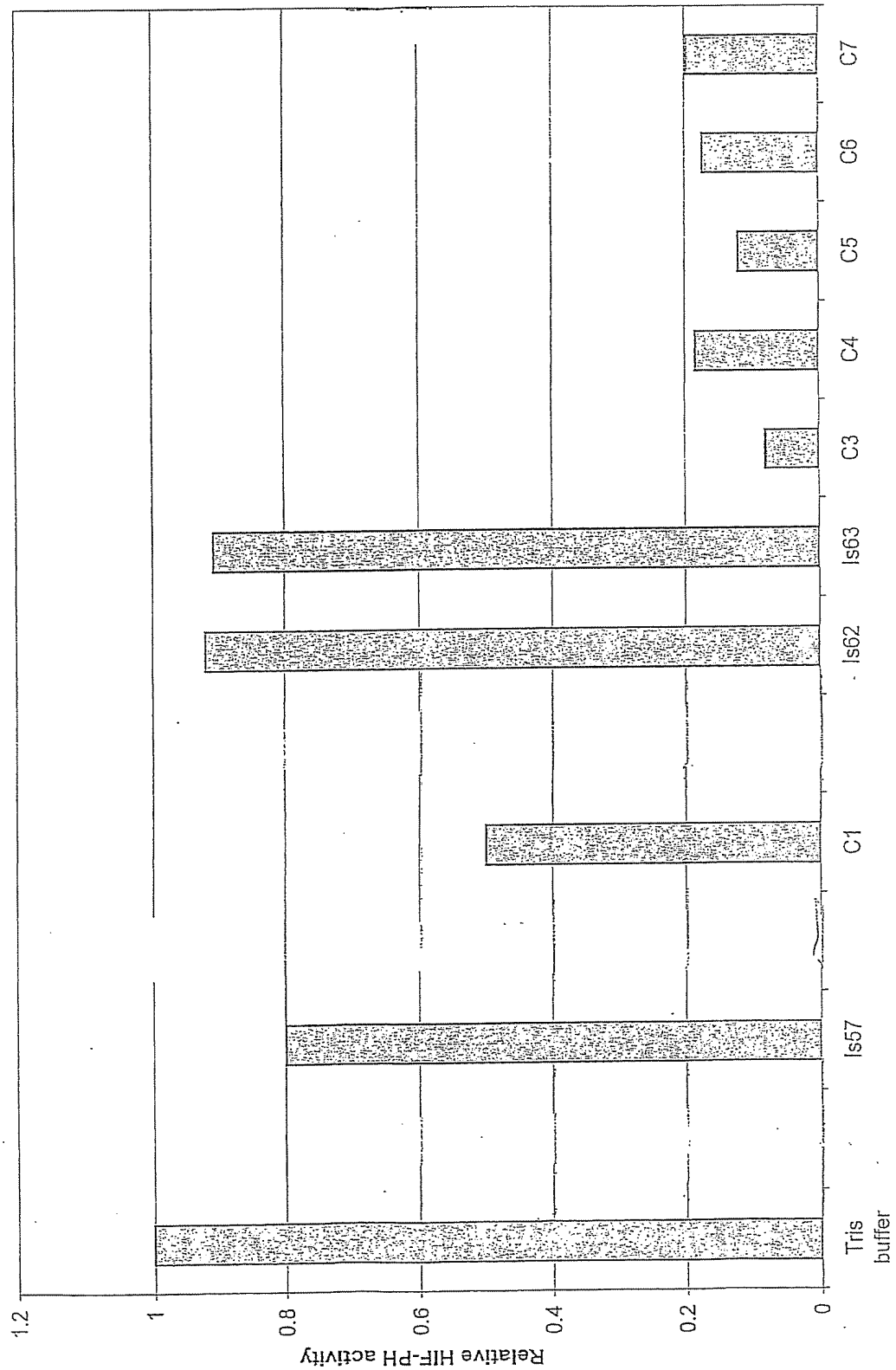
C12 Citric acid
C13
Is90

Figure 4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C5
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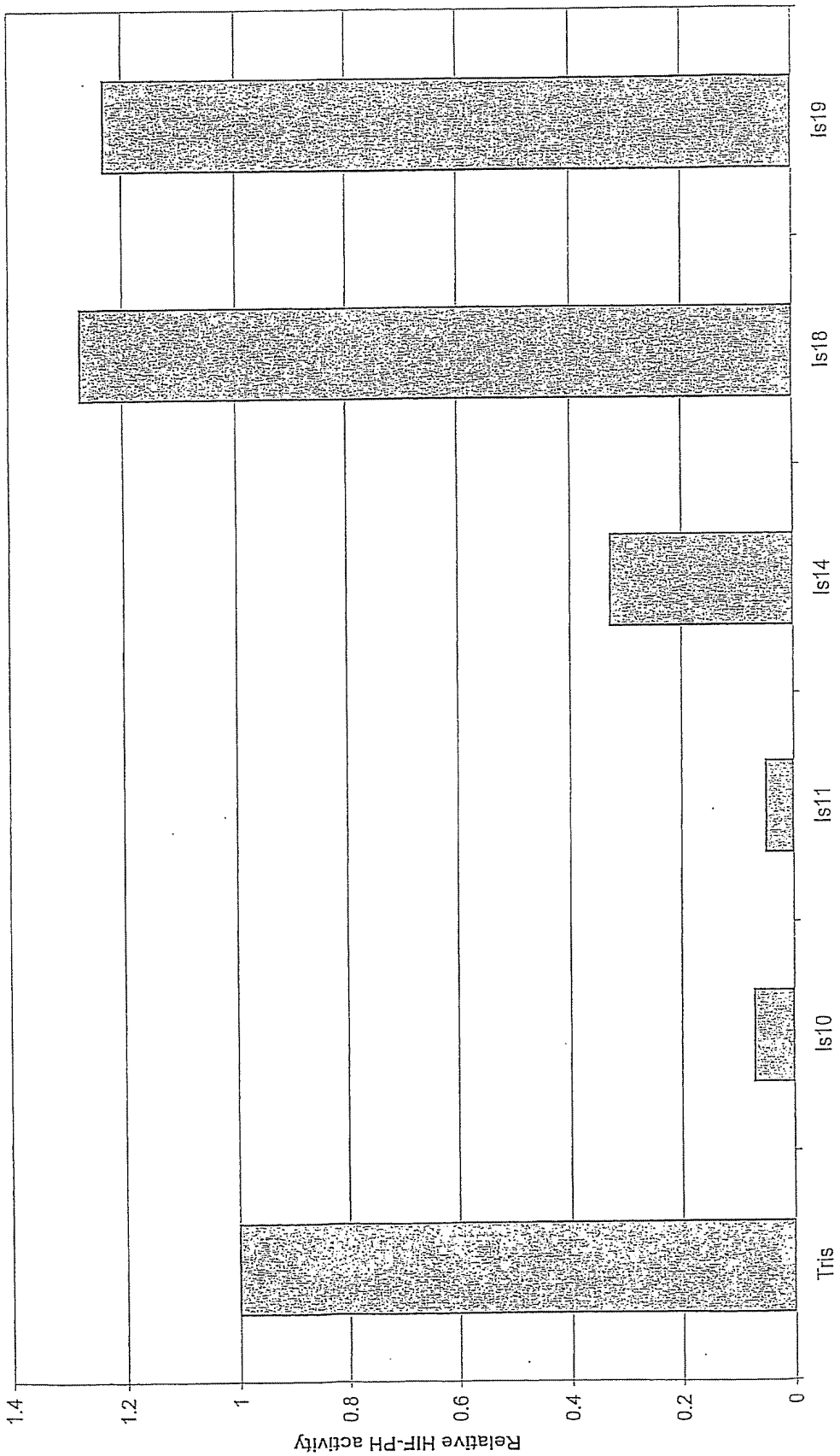
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Figure 5

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Gly Pro Gly Gly Pro Ser Pro Ser Glu Arg Asp Arg Gln Tyr Cys Glu
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ctg tgc ggg aag atg gag aac ctg ctg cgc tgc agc cgc tgc cgc agc      3270
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Ser Phe Tyr Cys Cys Lys Glu His Gln Arg Gln Asp Trp Lys Lys His
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Lys Leu Val Cys Gln Gly Ser Glu Gly Ala Leu Gly His Gly Val Gly
55                  60                  65                  70
cca cac cag cat tcc ggc ccc gcg ccg ccg gct gca gtg ccg ccg ccc     3414
Pro His Gln His Ser Gly Pro Ala Pro Pro Ala Ala Val Pro Pro Pro
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agg gcc ggg gcc cgg gag ccc agg aag gca gcg gcg cgc cgg gac aac     3462
Arg Ala Gly Ala Arg Glu Pro Arg Lys Ala Ala Ala Arg Arg Asp Asn
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gac cca gcg gcg gcc gcg tcg ccg tgt cgt gcg gcc gcc ggc ggc cag     3558
Asp Pro Ala Ala Ala Ala Ser Pro Cys Arg Ala Ala Ala Gly Gly Gln
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ggc tcg gcg gtg gct gcc gaa gcc gag ccc ggc aag gag gag ccg ccg     3606
Gly Ser Ala Val Ala Ala Glu Ala Glu Pro Gly Lys Glu Glu Pro Pro
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gcc cgc tca tcg ctg ttc cag gag aag gcg aac ctg tac ccc cca agc     3654
Ala Arg Ser Ser Leu Phe Gln Glu Lys Ala Asn Leu Tyr Pro Pro Ser
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Asn Thr Pro Gly Asp Ala Leu Ser Pro Gly Gly Gly Leu Arg Pro Asn
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Gly Gln Thr Lys Pro Leu Pro Ala Leu Lys Leu Ala Leu Glu Tyr Ile
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gtg ccg tgc atg aac aag cac ggc atc tgt gtg gtg gac gac ttc ctc     3798
Val Pro Cys Met Asn Lys His Gly Ile Cys Val Val Asp Asp Phe Leu
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ggc aag gag acc gga cag cag atc ggc gac gag gtg cgc gcc ctg cac     3846
Gly Lys Glu Thr Gly Gln Gln Ile Gly Asp Glu Val Arg Ala Leu His
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gac acc ggg aag ttc acg gac ggg cag ctg gtc agc cag aag agt gac     3894
Asp Thr Gly Lys Phe Thr Asp Gly Gln Leu Val Ser Gln Lys Ser Asp
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Ser Ser Lys Asp Ile Arg Gly Asp Lys Ile Thr Trp Ile Glu Gly Lys
            250                 255                 260
gag ccc ggc tgc gaa acc att ggg ctg ctc atg agc agc atg gac gac     3990
Glu Pro Gly Cys Glu Thr Ile Gly Leu Leu Met Ser Ser Met Asp Asp
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Leu Ile Arg His Cys Asn Gly Lys Leu Gly Ser Tyr Lys Ile Asn Gly
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Val Arg His Val Asp Asn Pro Asn Gly Asp Gly Arg Cys Val Thr Cys
                315                 320                 325
ata tat tat ctt aat aaa gac tgg gat gcc aag gta agt gga ggt ata     4182
Ile Tyr Tyr Leu Asn Lys Asp Trp Asp Ala Lys Val Ser Gly Gly Ile
            330                 335                 340
ctt cga att ttt cca gaa ggc aaa gcc cag ttt gct gac att gaa ccc     4230
Leu Arg Ile Phe Pro Glu Gly Lys Ala Gln Phe Ala Asp Ile Glu Pro
        345                 350                 355
aaa ttt gat aga ctg ctg ttt ttc tgg tct gac cgt cgc aac cct cat     4278
Lys Phe Asp Arg Leu Leu Phe Phe Trp Ser Asp Arg Arg Asn Pro His
    360                 365                 370
gaa gta caa cca gca tat gct aca agg tac gca ata act gtt tgg tat     4326
Glu Val Gln Pro Ala Tyr Ala Thr Arg Tyr Ala Ile Thr Val Trp Tyr
375                 380                 385                 390
ttt gat gca gat gag aga gca cga gct aaa gta aaa tat cta aca ggt     4374
Phe Asp Ala Asp Glu Arg Ala Arg Ala Lys Val Lys Tyr Leu Thr Gly
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gaa aaa ggt gtg agg gtt gaa ctc aat aaa cct tca gat tcg gtc ggt     4422
Glu Lys Gly Val Arg Val Glu Leu Asn Lys Pro Ser Asp Ser Val Gly
            410                 415                 420
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Lys Asp Val Phe
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Gln Asp Trp Lys Lys His Lys Leu Val Cys Gln Gly Ser Glu Gly Ala
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Leu Gly His Gly Val Gly Pro His Gln His Ser Gly Pro Ala Pro Pro
65                  70                  75                  80
Ala Ala Val Pro Pro Pro Arg Ala Gly Ala Arg Glu Pro Arg Lys Ala
                85                  90                  95
Ala Ala Arg Arg Asp Asn Ala Ser Gly Asp Ala Ala Lys Gly Lys Val
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Lys Ala Lys Pro Pro Ala Asp Pro Ala Ala Ala Ala Ser Pro Cys Arg
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Ala Ala Ala Gly Gly Gln Gly Ser Ala Val Ala Ala Glu Ala Glu Pro
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Gly Lys Glu Glu Pro Pro Ala Arg Ser Ser Leu Phe Gln Glu Lys Ala
145                 150                 155                 160
Asn Leu Tyr Pro Pro Ser Asn Thr Pro Gly Asp Ala Leu Ser Pro Gly
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Gly Gly Leu Arg Pro Asn Gly Gln Thr Lys Pro Leu Pro Ala Leu Lys
            180                 185                 190
Leu Ala Leu Glu Tyr Ile Val Pro Cys Met Asn Lys His Gly Ile Cys
        195                 200                 205
Val Val Asp Asp Phe Leu Gly Lys Glu Thr Gly Gln Gln Ile Gly Asp
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Glu Val Arg Ala Leu His Asp Thr Gly Lys Phe Thr Asp Gly Gln Leu
225                 230                 235                 240
Val Ser Gln Lys Ser Asp Ser Ser Lys Asp Ile Arg Gly Asp Lys Ile
                245                 250                 255
Thr Trp Ile Glu Gly Lys Glu Pro Gly Cys Glu Thr Ile Gly Leu Leu
            260                 265                 270
Met Ser Ser Met Asp Asp Leu Ile Arg His Cys Asn Gly Lys Leu Gly
        275                 280                 285
Ser Tyr Lys Ile Asn Gly Arg Thr Lys Ala Met Val Ala Cys Tyr Pro
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Gly Asn Gly Thr Gly Tyr Val Arg His Val Asp Asn Pro Asn Gly Asp
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Gly Arg Cys Val Thr Cys Ile Tyr Tyr Leu Asn Lys Asp Trp Asp Ala
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Lys Val Ser Gly Gly Ile Leu Arg Ile Phe Pro Glu Gly Lys Ala Gln
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Phe Ala Asp Ile Glu Pro Lys Phe Asp Arg Leu Leu Phe Phe Trp Ser
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Ala Ile Thr Val Trp Tyr Phe Asp Ala Asp Glu Arg Ala Arg Ala Lys
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Met Pro Leu Gly His Ile Met Arg Leu
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Asp Leu Glu Lys Ile Ala Leu Glu Tyr Ile Val Pro Cys Leu His Glu
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Cys Val Leu Glu Arg Val Lys Gln Leu His Cys Thr Gly Ala Leu Arg
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Asp Gly Gln Leu Ala Gly Pro Arg Ala Gly Val Ser Lys Arg His Leu
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Arg Gly Asp Gln Ile Thr Trp Ile Gly Gly Asn Glu Glu Gly Cys Glu
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gcc atc agc ttc ctc ctg tcc ctc atc gac agg ctg gtc ctc tac tgc 641
Ala Ile Ser Phe Leu Leu Ser Leu Ile Asp Arg Leu Val Leu Tyr Cys
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ggg agc cgg ctg ggc aaa tac tac gtc aag gag agg tct aag gca atg 689
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Glu Gly Lys Ser Phe Ile Ala Asp Val Glu Pro Ile Phe Asp Arg Leu
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ctg ttc ttc tgg tca gat cgt agg aac cca cac gaa gtg cag ccc tct      929
Leu Phe Phe Trp Ser Asp Arg Arg Asn Pro His Glu Val Gln Pro Ser
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Tyr Ala Thr Arg Tyr Ala Met Thr Val Trp Tyr Phe Asp Ala Glu Glu
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Arg Ala Glu Ala Lys Lys Lys Phe Arg Asn Leu Thr Arg Lys Thr Glu
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Gln Leu His Cys Thr Gly Ala Leu Arg Asp Gly Gln Leu Ala Gly Pro
50 55 60
Arg Ala Gly Val Ser Lys Arg His Leu Arg Gly Asp Gln Ile Thr Trp
65 70 75 80
Ile Gly Gly Asn Glu Glu Gly Cys Glu Ala Ile Ser Phe Leu Leu Ser
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Leu Ile Asp Arg Leu Val Leu Tyr Cys Gly Ser Arg Leu Gly Lys Tyr
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Tyr Val Lys Glu Arg Ser Lys Ala Met Val Ala Cys Tyr Pro Gly Asn
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Gly Thr Gly Tyr Val Arg His Val Asp Asn Pro Asn Gly Asp Gly Arg
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Cys Ile Thr Cys Ile Tyr Tyr Leu Asn Lys Asn Trp Asp Ala Lys Leu
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His Gly Gly Ile Leu Arg Ile Phe Pro Glu Gly Lys Ser Phe Ile Ala
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Asp Val Glu Pro Ile Phe Asp Arg Leu Leu Phe Phe Trp Ser Asp Arg
180 185 190
Arg Asn Pro His Glu Val Gln Pro Ser Tyr Ala Thr Arg Tyr Ala Met
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Thr Val Trp Tyr Phe Asp Ala Glu Glu Arg Ala Glu Ala Lys Lys Lys
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Phe Arg Asn Leu Thr Arg Lys Thr Glu Ser Ala Leu Thr Glu Asp
225 230 235